



ELSEVIER

respiratoryMEDICINE 

Diagnostic accuracy of bronchoalveolar lavage samples in immunosuppressed patients with suspected pneumonia: Analysis of a protocol

Lázaro Vélez^{a,d,*}, Luz Teresita Correa^d, Maria Angélica Maya^d,
Patricia Mejía^d, Jorge Ortega^b, Victoria Bedoya^c, Héctor Ortega^b

^aSección de Enfermedades Infecciosas, Departamento de Medicina Interna, Hospital Universitario San Vicente de Paúl and Universidad de Antioquia. Sede de Investigación Universitaria, Calle 62 #52–59, Laboratorio 630, Medellín, Colombia

^bSección de Neumología, Departamento de Medicina Interna, Hospital Universitario San Vicente de Paúl and Universidad de Antioquia. Sede de Investigación Universitaria, Calle 62 #52–59, Laboratorio 630, Medellín, Colombia

^cGrupo de Inmunovirología, Departamento de Medicina Interna, Hospital Universitario San Vicente de Paúl and Universidad de Antioquia. Sede de Investigación Universitaria, Calle 62 #52–59, Laboratorio 630, Medellín, Colombia

^dGrupo Investigador de Problemas en Enfermedades Infecciosas (GRIPE), Departamento de Medicina Interna, Hospital Universitario San Vicente de Paúl and Universidad de Antioquia. Sede de Investigación Universitaria, Calle 62 #52–59, Laboratorio 630, Medellín, Colombia.

Received 10 January 2007; accepted 7 May 2007

Available online 12 July 2007

KEYWORDS

Bronchoalveolar lavage;
Immunocompromised patients;
Pneumonia;
Diagnostic accuracy

Summary

Background: Fast and accurate etiologic diagnosis of pneumonia in immunocompromised patients is essential for a good outcome. Utility of bronchoalveolar lavage (BAL) samples has already been established, but studies about them are scarce and limited to few countries. We aimed to evaluate the accuracy of a diagnostic protocol, emphasizing on local epidemiology, rapidity, and yield of different techniques.

Methods: One year prospective study of 101 consecutive immunosuppressed patients admitted with suspected pneumonia to a university hospital. They all had bronchoscopic BAL ($n = 109$) and respiratory sampling. Conventional microbiological studies, cytomegalovirus pp65 antigenemia and transbronchial biopsy (TBB), whenever considered pertinent,

Abbreviations: BAL, bronchoalveolar lavage; HUSVP, Hospital Universitario San Vicente de Paúl; TBB, transbronchial biopsy; FB, flexible bronchoscopy; ZN, Ziehl-Neelsen; TBO, modified toluidine blue; CFU, colony forming units; CMV, Cytomegalovirus; UAIC, unspecific airways inflammatory condition; PPV, positive predictive value; NPV, negative predictive value; TB, tuberculosis; PCR, polymerase chain reaction; CSF, cerebrospinal fluid

*Corresponding author. Tel.: +57 42106542; fax: +57 45129664.

E-mail addresses: clamona@une.net.co (L. Vélez), correamar@une.net.co (L.T. Correa), mangelica@une.net.co (M.A. Maya), pamegi16@hotmail.com (P. Mejía), aggt@une.net.co (J. Ortega), vibe@elhospital.org.co (V. Bedoya), horteiga@une.net.co (H. Ortega).

were done. Results were analyzed along with other diagnostic procedures, clinical course and final outcome.

Results: HIV/AIDS infection was the most frequent cause of inclusion ($n = 80$). Infections accounted for 79 out of 122 final diagnoses (64.8%). Our protocol identified 60 infectious and 3 noninfectious pathologies (general yield: 51.6%). Sensitivity in pulmonary infections was 75.9% (IC95%: 64.8–84.6%), specificity 86.0% (72.6–93.7%), positive predictive value 89.6% (79.1–95.3%), negative predictive value 69.4% (56.2–80.1%), accuracy 79.8% (71.7–86.2%). *Mycobacterium* spp. ($n = 27$), bacteria ($n = 19$), *Pneumocystis jirovecii* ($n = 18$) and other fungi (histoplasmosis: 6, aspergillosis: 5, cryptococcosis: 3) were the most common infectious pathogens. Direct microscopy allowed an early definite/presumptive diagnosis in 36/49 fungal and mycobacterial infections (73.5%). Up to 30% of mycobacterial infections were missed.

Conclusions: Systematical study of BAL samples has a high diagnostic yield in our immunocompromised patients with suspected pneumonia. As economical and epidemiological conditions of regions are different, it should be tried everywhere.

© 2007 Elsevier Ltd. All rights reserved.

Introduction

Pneumonia is a major cause of morbidity and mortality in immunosuppressed patients.^{1–3} Rapid diagnosis and early treatment are necessary for a good outcome.^{1,4} However, accurate microbiological diagnosis is challenging as many infectious and noninfectious conditions have comparable clinical presentation.^{1,2,5–8}

Flexible bronchoscopy (FB) with bronchoalveolar lavage (BAL) is simple, safe, fast and reliable. It has been extensively used as diagnostic procedure for assessing immunosuppressed hosts with pulmonary infiltrates.^{7,9–14} BAL sensitivity and specificity are comparable to other techniques.¹⁵ Ideally, a clinician should submit BAL samples to a single microbiology laboratory, results of histochemical stains should be available within hours of specimen submission, and a report summarizing all of the information obtained from analysis should be issued from that laboratory.⁹ Several groups have already demonstrated the clinical value of BAL protocols, particularly when samples are methodically studied.^{7,9,10,16–18} However, they are limited to few countries.

Local data in Latin America, specifically in Colombia, are scarce.^{19,20} This study was conducted to evaluate the role of systematic analysis of BAL samples in the etiologic assessment of immunosuppressed patients with pneumonia, in a region with different social, economical and epidemiological patterns. In order to optimize the process, and elaborate guidelines for empirical treatment in these cases, we emphasized on the protocol diagnostic accuracy, rapidity to preliminary diagnosis, specific problems with some pathogens, and differences with other countries reports.

Materials and methods

Patient population

We included 101 immunosuppressed patients consecutively admitted to Hospital Universitario San Vicente de Paúl (HUSVP, Medellín-Colombia) with suspected pneumonia from June 2000 to July 2001. Patients older than 12 years were

included if they had at least one of the following symptoms: cough, dyspnea, abnormal auscultatory findings and new pulmonary infiltrates. Immunosuppression was considered if: HIV infection or AIDS; neutropenia <500 cells/mm³, bone marrow or solid organ transplantation, hematological malignancies, immunosuppressive treatment (prednisone 0.3 mg/kg/d, or its equivalent, longer than 2 weeks), noncontrolled diabetes and splenectomy. The research team had no control over treatment. All patients signed an informed consent according to legal requirements (Colombia Ministry of Health Resolution 008430, 1993), and the study was approved by University of Antioquia Ethics Committee and HUSVP Internal Review Board. Exclusion criteria were pregnancy, severe heart arrhythmia, serious hemoptysis, instable hemodynamic status, critical respiratory failure ($\text{PaO}_2/\text{FiO}_2 < 120$) and any other condition with risk for bronchoscopic procedures.

Bronchoalveolar lavage and respiratory sampling

FB and BAL were performed following American Thoracic Society guidelines.²¹ Transbronchial biopsy (TBB) was done in case of diffuse interstitial or reticulonodular infiltrates (at pulmonologist's discretion), whenever the patient had no contraindications for it (platelet count $\leq 50,000$ /mm³, increased coagulation times, significant pulmonary hypertension, or poor functional respiratory reserve). An open-lung biopsy was carried out if BAL studies were no diagnostic and clinical conditions allowed it.

Laboratory processing of specimens

Each specimen was quantitatively cultured for bacteria on conventional agar media. All isolates containing $\geq 10^3$ colony forming units (CFU)/ml were identified to specie. In general, BAL specimens were considered significant if they contained $\geq 10^4$ CFU and $\leq 1\%$ squamous epithelial cells in Wright-stained slides.^{9,22–24} After quantitative cultures were set up, specimens were centrifuged at 1500g for 20 min at 4 °C. Pellets were partitioned. A portion was plated for fungus (incubated at 30 °C in aerobic conditions for 4 weeks

on Mycosel, Girasol, and Sabouraud's agar), and mycobacteria (at 37 °C in aerobic conditions for 6 weeks on Ogawa-Kudoh medium and thin layer agar²⁵). The other portion was suspended in 4 ml of Hanks salt solution and cytocentrifuged at 1500 rpm for 15 min (Cytospin III, Shandon Instruments, Sewickley, PA) after adjusting to 10⁵ cells/ml. Slides were stained with Wright, Gram, Ziehl-Neelsen (ZN), modified Kinyoun and modified toluidine blue (TBO), according to Kahn-Jones' protocol.⁹

Bronchial brushings, transbronchial and open lung biopsy specimens were stained with hematoxylin-eosin, ZN and Gomori-methenamine silver.

Variables recorded

Conventional clinical and laboratory data with special emphasis on type, stage and severity of immunosuppression, antibiotic's use within previous week of respiratory sampling, and microbiological analysis of blood, sputum, urine, pleural and spinal fluids. Cytomegalovirus (CMV) pp65 antigenemia assay was evaluated in all cases.

All in-patients were followed until discharge or death. Survivors were followed up for 1 year through visits to the infectious diseases clinic and/or by reviewing their clinical charts. Telephone calls to patients, their families and doctors in charge were tried when they did not return to the hospital.

Diagnostic criteria

To determine whether an identified microorganism on BAL was a true pathogen, we applied strict diagnostic criteria (Table 1). Besides that, etiology was established based on other diagnostic procedures, clinical course and final outcome. At least two of the clinical investigators evaluated the information of every case. Unspecific airways inflammatory condition (UAIC) was defined by the presence of: (i) cough without dyspnea, (ii) unspecific auscultatory findings in absence of pulmonary infiltrates, (iii) spontaneous improvement without specific treatment, and (iv) no microorganisms isolated. Diagnoses of pulmonary fibrosis, malignancies, pulmonary hypertension or edema were based on clinical, radiographical and histopathological evaluation. If patients had pulmonary infiltrates, but histopathological and microbiological results were not conclusive, final diagnosis was classified as unclear.

Statistical analysis

Data were collected and analyzed using Epiinfo software, version 6,04 (Epidemiology Program Office, CDC, Atlanta, GA). Two-tailed Fisher's exact test was used to compare pairs of proportions. A *P*-value < 0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values (PPV-NPV), and accuracy (a+d/a+b+c+d)²⁷ were calculated using Epidat software, version 2,0 (Xunta de Galicia, Santiago de Compostela, España).

Table 1 Criteria to determine whether an identified infectious agent on BAL was a true pathogen.^{7,10,15,16,26}

- 1 Any positive stain and/or isolation of *P. jirovecii*, *M. tuberculosis* and *H. capsulatum*.
- 2 Isolation of any recognized bacterial pulmonary pathogen on a quantitative culture $\geq 10^4$ CFU/ml and epithelial squamous cells $\leq 1\%$. Also, any bacterial growth was considered significant in patients who were on appropriate antibiotics for the isolated bacteria within the previous week, as long as the Gram stain was compatible and no other etiology was demonstrated. If culture was negative but the patient was on broad spectrum antibiotics when BAL was performed, bacteria were considered probable cause of pneumonia, as long as he/she improved with, and no other causes accounted for symptoms.
- 3 Isolation of *Cryptococcus neoformans* on BAL, when: (i) it was isolated concurrently in other sterile body fluid, such as blood or CSF; (ii) no other possible pulmonary pathogen was isolated; or (iii) there was tissue infiltration on histopathology.
- 4 Isolation of *Aspergillus* spp. in case of: (i) neutropenia or hematological malignancies; (ii) hemoptysis or alveolar hemorrhage; (iii) concurrent isolation in other sterile body fluid, such as pleural fluid; or (iv) tissue infiltration on pulmonary biopsy.
- 5 Isolation of any nontuberculous mycobacteria when there was no other pathogen accounting for the pneumonia.
- 6 Histopathological evidence of pulmonary damage due to invasive CMV or *Candida* spp.

BAL: bronchoalveolar lavage; CFU: colony forming units; CSF: cerebrospinal fluid; CMV: Cytomegalovirus.

Results

Patients

During the study, 109 BAL procedures were performed in 101 patients. Table 2 displays basal features of them. HIV infection was the cause of inclusion in 80 patients, 90% with AIDS.²⁸ Those with two or more causes of immunosuppression were included in the most relevant category at time BAL was done.

Etiology of pulmonary symptoms

Table 3 shows the distribution of 122 final diagnoses. The most common etiologies were mycobacteria (26.7%), UAIC (23.8%, almost exclusively in AIDS patients), bacteria (18.8%) and *Pneumocystis jirovecii* (17.8%). Predominant etiology varied according to immunosuppression: *Mycobacterium* spp. (30%), UAIC (28.7%) and *P. jirovecii* (21.2%) in HIV/AIDS patients, aspergillosis (27.3%) in transplantation, and bacterial pneumonia (70%) in the others.

Table 2 Baseline characteristics of 101 immunosuppressed patients with suspected pneumonia.

Age (years)	
Mean \pm SD* (range)	34.1 \pm 10.8 (13–75)
Sex	
Male/female (%)	74/26
Type of immunosuppression	
(1) HIV/AIDS, n (%)	80 (79%)
On HAART,** n (%)	12 (15%)
T CD4+ lymphocytes count (n = 47)	
Mean, cells/mm ³ \pm SD (range)	128 \pm 211 (2–1303)
Median (cells/mm ³)	65
CD4+ \leq 200, n (%)	39 (83%)
CD4+ > 200, n (%)	8 (17%)
Viral load (n = 45)	
Mean, copies/mm ³ \pm SD	313,241 \pm 282,914
< 10,000, n (%)	3 (6.7%)
\geq 10,000, n (%)	42 (93.3%)
(2) Transplantation patients, n (%)	11 (11%)
Kidney, n	10
Bone marrow, n	1
Days after transplantation, mean \pm SD (range)	970 \pm 1187 (50–4342)
Median (days)	611
(3) Others, n (%)	10 (10%)
Hematological malignancies, [†] n	6
Corticosteroids therapy, n	3
Neutropenia, [‡] n	1

*SD: standard deviation.

**HAART: highly active antiretroviral therapy.

[†]Hematological malignancies: Hodgkin disease (2 patients), non-Hodgkin lymphoma (1), acute myeloid leukemia (1), acute lymphoid leukemia (1), multiple myeloma (1).[‡]Secondary to gastrointestinal sepsis.

Diagnostic accuracy of the protocol

Infections accounted for 79/122 (64.8%) final diagnoses (Table 3). BAL samples allowed us to identify 97 agents, 60 of them considered true pathogens. *Candida* spp. and bacteria were common colonizers (Fig. 1). Only 3 of 43 noninfectious pulmonary pathologies were diagnosed by the protocol, all of them by TBB (Fig. 2). Thus, general yield was 51.6% (63/122). In seven cases, microorganisms initially identified on BAL as pathogens, were considered misclassified by further analysis. According to Table 1, sensitivity in pulmonary infections was 75.9% (IC95%: 64.8–84.6%), specificity 86.0% (72.6–93.7%), PPV 89.6% (79.1–95.3%), NPV 69.4% (56.2–80.1%) and accuracy 79.8% (71.7–86.2%). Sensitivity was 100% for *P. jirovecii* (18/18), histoplasmosis (6/6) and cryptococcosis (3/3), 70.4% for mycobacteria (19/27), 60% for aspergillosis (3/5) and 57.9% for conventional bacteria (11/19) (Fig. 2). Direct microscopy was positive in 36 of 49 patients (73.5%) with fungal and/or mycobacterial pneumonia (*P. jirovecii* 18/18, mycobacteria

10/19, histoplasmosis 3/6, aspergillosis 3/3 and cryptococcosis 2/3).

Mycobacteria were considered the cause of pneumonia in 27 patients, but this protocol only diagnosed 19:12 *M. tuberculosis*, 4 nontuberculous mycobacteria and 3 ZN-positive, but culture-negative cases. Of eight patients missed by the protocol, two had pulmonary tuberculosis (TB) and six had extrapulmonary forms (two mediastinitis, one pericarditis, one pleuritis, one lymphadenitis and one gastrointestinal TB). Three were already on tuberculosis treatment. Sensitivity of cultures and acid fast staining in BAL samples was 59% (16/27) and 37% (10/27), respectively; 13 of 16 positive cultures grew in thin layer, 13 in Ogawa and 10 in both media. Time required to growth was 22 \pm 10.7 days in Ogawa (range: 12–55; median: 20) and 14.3 \pm 6.0 in thin layer (range: 4–26; median: 15).

BAL samples allowed to detect 11/19 patients with bacterial pneumonia (57.9%). All cases not diagnosed by BAL corresponded to partially treated infections (mean: 6 days, range: 2–12), which improved with prescribed antibiotics.

Histopathological studies were performed in 28 patients: 24 TBB, 3 open lung biopsies and 1 necropsy. TBB identified 13 of 23 infectious agents (56.5%) demonstrated in these 24 patients. Sensitivity was 50% (5/10) for mycobacteria, 33% (2/6) for bacteria, 100% for *P. jirovecii* (3/3), and 75% for other fungal infections (3/4).

Diagnostic fails

This protocol failed as a diagnostic tool in 59/122 (48.4%) final conditions (Fig. 2); 19 (32%) were attributed to infectious diseases (8 by bacteria, 8 mycobacteria, 2 aspergillus, 1 CMV), and 36 (61%) to noninfectious pathologies (24 cases of UAIC, 5 fibrosis, 4 malignancies, 2 edema and 1 case due to pulmonary hypertension). It was impossible to define the cause of pneumonia in 4 patients (7%).

Diagnostic yield of other procedures

CMV disease was demonstrated in seven patients (six AIDS and one kidney transplant recipient). Of them, three had retinitis, three had colitis (by biopsy) and one, the transplantation case, had pneumonia associated to central nervous system involvement (by necropsy). Among these patients, antigenemia assay was negative in 4, low-positive in 2 (<10 positive cells/150,000 leukocytes) and highly positive (180/150,000) in 1 case (CMV ulcerative colitis). On the other hand, only 1 of 13 patients with pp65 \geq 10 cells/150,000 leukocytes had CMV disease.

Chest X-rays had no significant value to predict the etiology of infection.

Discussion

Systematical study of BAL samples leads to accurate and opportune diagnosis of etiology in immunosuppressed patients with suspected pneumonia.^{1–4,29} We found a general yield of 51.6%, but 75.9% in infectious diseases. These results are very similar to previous reports from

Table 3 Etiology of 122 final diagnoses in 109 BAL procedures made to 101 patients, according to type of immunosuppression.

Final diagnosis, <i>n</i> (%)	HIV/AIDS (<i>n</i> = 80)	Transplant (<i>n</i> = 11)	Others* (<i>n</i> = 10)	Total (<i>n</i> = 101)
Mycobacterial infection**	24 (30.0)	2 (18.2)	1 (10.0)	27 (26.7)
UAIC†	23 (28.7)	1 (9.1)	0	24 (23.8)
Bacterial pneumonia	11 (13.7)	1 (9.1)	7 (70.0)	19 (18.8)
<i>P. jirovecii</i>	17 (21.2)	1 (9.1)	0	18 (17.8)
Histoplasmosis	5 (6.2)	1 (9.1)	0	6 (5.9)
Pulmonary fibrosis	3 (3.7)	2 (18.2)	1 (10.0)	6 (5.9)
Aspergillosis	0	3 (27.3)	2 (20.0)	5 (4.9)
Unclear cause	2 (2.5)	2 (18.2)	0	4 (4.0)
Malignancy infiltration‡	1 (1.2)	0	3 (30.0)	4 (4.0)
Pulmonary edema	2 (2.5)	2 (18.2)	0	4 (4.0)
Cryptococcosis	3 (3.7)	0	0	3 (3.0)
Pulmonary hypertension	1 (1.2)	0	0	1 (1.0)
Cytomegalovirus	0	1 (9.1)	0	1 (1.0)
Total diagnoses, § <i>n</i>	92	16	14	122
Infections, <i>n</i> (%)	60 (65.2)	9 (56.2)	10 (71.4)	79 (64.8)

*Others: hematological malignancies (6 patients), on therapy with corticosteroids (3), neutropenic patient (1).

**Mycobacterial infections: tuberculosis (19 patients), nontuberculous mycobacteria (4), culture negative cases (4).

†UAIC: unspecific airways inflammatory condition (see "Diagnostic criteria" in Materials and Methods).

‡Malignancy infiltration of lung: Kaposi sarcoma (1 case in a HIV/AIDS patient), anaplastic lymphoma (1), Hodgkin disease (1), multiple myeloma (1).

§Total diagnoses: the sum of each column is greater than the number of patients in each category as several patients had more than one diagnosis.

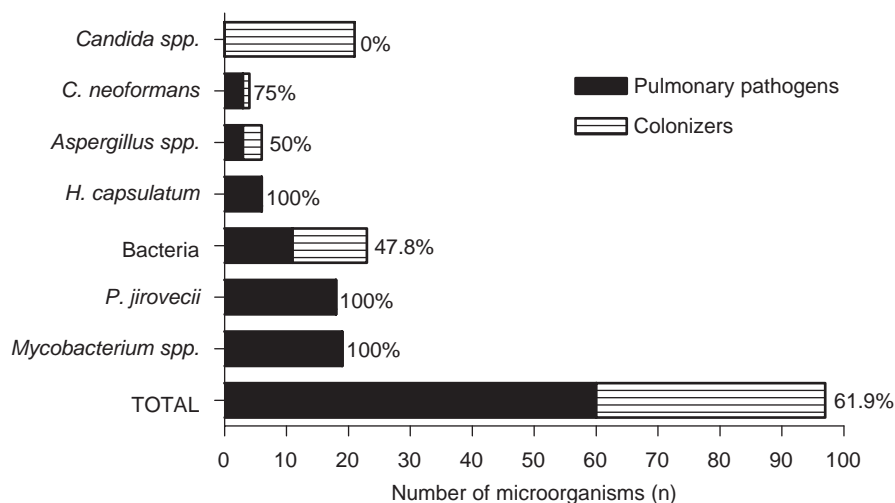


Figure 1 Distribution of the different germs isolated in BAL samples (*n* = 109) in 101 immunosuppressed patients with suspected pneumonia. Black bars correspond to microorganisms considered true pulmonary pathogens, and striped bars to colonizers, according to criteria displayed in Table 1. Numbers in front of bars represent the percentage of each isolated microorganism considered true pathogen.

United States^{7,9,10,14} and Spain,^{16,17} whose general yield was 51–60%; for pulmonary infections was 52–81%, and the proportion due to infectious diseases 61–72% (65% in our study).

Distribution of pulmonary pathogens depends on the epidemiology of each region. In this study, *Mycobacterium* spp., mainly tuberculosis, affected one of every four

immunosuppressed patients with pneumonia, unlike developed countries, where it involves 2–4% of them.^{7,9,10,16,17} It explained one-third of infections identified in this study (27/79, 34.2%). Previous studies in Colombia also pointed TB as the most frequent opportunistic infection, complicating 15–25% of AIDS patients.^{19,30} TB represents a serious public health threat in our country (incidence among 25–49³¹ and

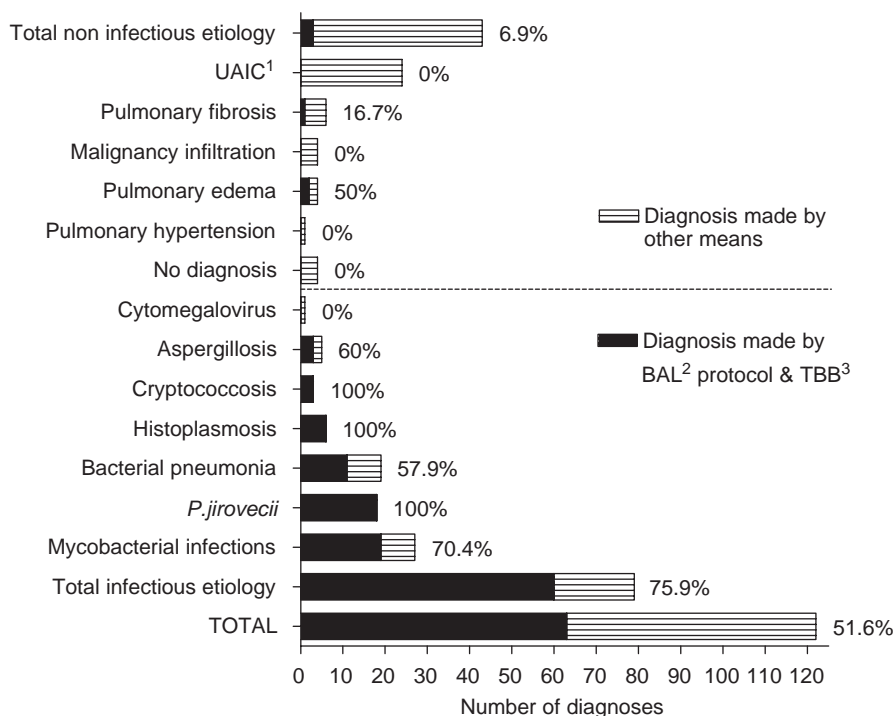


Figure 2 Definitive diagnoses in 101 immunosuppressed patients with suspected pneumonia. Black bars correspond to 63 diagnoses made by our protocol (including TBB when it was considered pertinent); striped bars represent 59 diagnoses in which the protocol was of no help. Total number of diagnoses was greater than 101 as several patients have more than one diagnosis. Figures in front of bars are the percentage of every diagnosis made by our protocol: (1) unspecific airways inflammatory condition; (2) bronchoalveolar lavage; (3) transbronchial biopsy.

50–84/100,000³²) and all tropical Latin American region.³² Furthermore, the magnitude of the problem can be even worse because diagnosis is still elusive (the protocol missed 8/27 mycobacterial infections, 29.6%). There are several possible explanations for these false negative cases: (i) 3/8 were on TB treatment when BAL was carried out (on the 1st, 2nd and 5th month, respectively) and (ii) presence of interstitial more than alveolar involvement. Unfortunately, many clinicians do not use to culture pulmonary tissue; and (iii) we did not make TBB to everyone in the study. However, histopathological yield for mycobacteria was only 50%. Our results highlight the importance of other diagnostic tools, such as blood/tissue cultures and polymerase chain reaction (PCR) in BAL samples. Meanwhile, in high prevalence regions, clinicians should consider the empirical use of TB treatment, especially when no other pathogen is found responsible.

Frequency of histoplasmosis was similar to reports from USA in HIV patients,¹⁰ but greater than in Spain¹⁶ and non-HIV immunocompromised hosts.^{9,14,17} On the other hand, incidence of bacterial pneumonia (17%) and *P. jirovecii* (16%) were within percentages reported elsewhere (7–27% and 2–47%); however, the occurrence of bacteria in our protocol (17%) could be underestimated by use of broad spectrum antibiotics before processing BAL samples.^{22,33} In fact, all eight patients with bacterial pneumonia, whose diagnosis was missed, were on antibiotics at the time of BAL (mean: 6 days).

Direct stains from most patients with fungal and mycobacterial infections were reported as positive within

the first 6 h after BAL (36/49, 73.5%). These preliminary results were timely enough to start early treatment. The faster growth of mycobacteria in thin layer agar than in Ogawa medium sped up 1 week identification and susceptibility testing of mycobacteria.

Noninfectious diseases explained most of missed diagnosis, as has also been reported elsewhere⁷ (Fig. 2). UAIC was the most common cause in our cases; it occurred in patients with decreased level of consciousness, whose impossibility to deal with bronchial secretions gives them cough and abnormal auscultatory findings. Most had opportunistic AIDS-related infections (such as CMV or toxoplasma encephalitis) and severe AIDS–dementia complex. They all got better after improving their respiratory hygiene and neurological conditions.

Our study had two important limitations. First, we did not include diagnostic assays to rule out the presence of “atypical” germs and/or respiratory viruses. According to previous studies,^{9,16,17,34} pneumonia could be caused by these agents in 17–19% of immunosuppressed patients. Nevertheless, several facts suggest it should not be a problem in our population. First, it is known that CMV is not a frequent cause of pneumonia in HIV/AIDS patients, who represented the majority of individuals involved (80/101).¹⁶ Secondly, *Legionella* spp. and *Chlamydomphila pneumoniae* do not seem to be a big problem in South America, although information about them is limited³⁵; and *Mycoplasma pneumoniae* is not especially common in these patients.^{16,17} Finally, there was not a documented

Colombian outbreak of viral infections in population older than 12 years during the study.³⁶ Although it is still possible that any of these agents explained some cases, our data suggest that they would have no significant impact on the results.

A second limitation is the lack of a reliable "gold standard" test in this setting, limitation shared by all prior studies. However, we believe that, as usually done in clinical practice, reaching the final diagnosis based on strict criteria, including clinical course and outcome, allows us to provide useful data about the diagnostic accuracy of our protocol, main goal of the study. On the other hand, it would have been very useful to determine the impact of the protocol on time to recovery, morbidity and mortality. But our design, exclusively conceived to determine etiology, does not provide an answer to that question. That is something that will have to be done in the next future.

CMV antigenemia assay did not seem to play any important role in our patients. It has been described an association between high systemic CMV load and CMV disease in HIV-infected individuals and solid-organ transplantation, but correlation is inconsistent, and some patients with low/undetectable systemic viral load can develop CMV disease.³⁷ Although antigenemia assay has shown good results in some immunosuppressed patients and several institutions including ours,^{38,39} these results support that other factors like viral virulence and tropism, dissemination ability of certain genotypes, and CMV-specific immunity may be important for the development of CMV disease.³⁷ To determine its role in these patients, it would have been necessary to measure CMV load in BAL samples and correlate these findings with those coming from traditional and shell vials cultures.

Our findings point to some issues that have to be studied in future research. First, it is required to clarify the real role of atypical germs and respiratory viruses in immunocompromised patients, and the contribution of microbiological cultures of TBB and PCR testing in BAL samples for diagnosing mycobacterial and fungal diseases. Second, because an invasive procedure such as a FB may be unsafe in some critically ill patients, it is important to evaluate the diagnostic accuracy of nonbronchoscopic BAL under these circumstances.

In conclusion, our findings highlight that as economical and epidemiological conditions of regions are different, systematical study of immunosuppressed patients with suspected pneumonia should be tried everywhere. Opportune and accurate results are very useful for affected patients, clinicians, insurance companies and health authorities involved in care-giving activities and local guidelines development. Furthermore, in high incidence regions of tuberculosis, rapid identification and treatment of potential sources will allow better control of this kind of nosocomial-acquired infections.

Conflict of interest

Lázaro Vélez has received research funding from Astra-Zeneca and Roche Colombia, and has been a consultant for Pfizer. Other authors did not declare conflict of interest.

Acknowledgments

The authors thank to the Bacteriology and Mycology Section, Department of Microbiology and Parasitology, Universidad de Antioquia and to Hospital Universitario San Vicente de Paúl (HUSVP), for the logistics support to the project; to Héctor Iván García and Jesús Ochoa for their statistical assistance, and to Gloria Durango, mycologist, for her collaboration through all the study.

Financial support: This study was supported by Universidad de Antioquia, Medellín, Colombia (contract 9955); Productos Roche (Colombia) supported diagnostic kits for Cytomegalovirus (CMV) pp65 antigen assay.

References

1. Rubin RH, Greene R. Clinical approach to the compromised host with fever and pulmonary infiltrates. In: Rubin RH, editor. *Clinical approach to infection in the compromised host*. 3rd ed. New York: Plenum Publishing Corporation; 1994. p. 121–61.
2. Cunha BA. Pneumonias in the compromised host. *Infect Dis Clin North Am* 2001;15:591–612.
3. Collin BA, Ramphal R. Pneumonia in the compromised host including cancer patients and transplant patients. *Infect Dis Clin North Am* 1998;12:781–805.
4. Raño A, Agustí C, Benito N, et al. Prognostic factors of non-HIV immunocompromised patients with pulmonary infiltrates. *Chest* 2002;122:253–61.
5. Marrie TJ. Community-acquired pneumonia. *Clin Infect Dis* 1994;18:501–13.
6. American Thoracic Society. Guidelines for the management of adults with community-acquired pneumonia: diagnosis, assessment of severity, antimicrobial therapy and prevention. *Am J Respir Crit Care Med* 2001;163:1730–54.
7. Pisani RJ, Wright AJ. Clinical utility of bronchoalveolar lavage in immunocompromised hosts. *Mayo Clin Proc* 1992;67:221–7.
8. Crawford SW. Non-infectious lung disease in the immunocompromised host. *Respiration* 1999;66:385–95.
9. Kahn FW, Jones JM. Analysis of bronchoalveolar lavage specimens from immunocompromised patients with a protocol applicable in the microbiology laboratory. *J Clin Microbiol* 1988;26:1150–5.
10. Baughman RP, Dohn MN, Frame PT. The continuing utility of bronchoalveolar lavage to diagnose opportunistic infection in AIDS patients. *Am J Med* 1994;97:515–22.
11. Hohenadel IA, Kiwor M, Genitsariotis R, et al. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broad spectrum antibiotic and antifungal regimen. *Thorax* 2001;56:115–20.
12. Reichenberger F, Dickenmann M, Binet I, et al. Diagnostic yield of bronchoalveolar lavage following renal transplantation. *Transpl Infect Dis* 2001;3:2–7.
13. Murray PV, O'Brien ME, Padhani AR, et al. Use of first line bronchoalveolar lavage in the immunosuppressed oncology patient. *Bone Marrow Transpl* 2001;27:967–71.
14. Jain P, Sandur S, Meli Y, et al. Role of flexible bronchoscopy in immunocompromised patients with lung infiltrates. *Chest* 2004;125:712–22.
15. Torres A, El-Ebiary M. Invasive diagnostic techniques for pneumonia: protected specimen brush, bronchoalveolar lavage, and lung biopsy methods. *Infect Dis Clin North Am* 1998;12:701–22.
16. Danés C, González-Martín J, Pumarola T, et al. Pulmonary infiltrates in immunosuppressed patients: analysis of a diagnostic protocol. *J Clin Microbiol* 2002;40:2134–40.

17. Rañó A, Agustí C, Jimenez P, et al. Pulmonary infiltrates in non-HIV immunocompromised patients: a diagnostic approach using non-invasive and bronchoscopic procedures. *Thorax* 2001;56: 379–87.
18. Carroll KC. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. *J Clin Microbiol* 2002; 40:3115–20.
19. Estrada S, González MA, Giraldo AM, et al. Etiología de la neumopatía en pacientes con SIDA. *Acta Méd Colomb* 1993; 18:164–71.
20. Reyes L, Sussmann O, Rivas P, et al. Cultivos cuantitativos e infiltrados pulmonares en pacientes con cáncer. *Rev Colomb Neumol* 1998;10:157–64.
21. American Thoracic Society. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. *Am Rev Respir Dis* 1990;142:481–6.
22. Thomson RB. Laboratory diagnosis of respiratory infections. *Curr Opin Infect Dis* 1999;12:115–9.
23. Jourdain B, Joly-Guillou ML, Dombret MC, et al. Usefulness of quantitative cultures of BAL fluid for diagnosing nosocomial pneumonia in ventilated patients. *Chest* 1997;111:411–8.
24. Meduri GU, Baselski V. The role of bronchoalveolar lavage in diagnosing nonopportunistic bacterial pneumonia. *Chest* 1991; 100:179–90.
25. Mejía GI, Castrillón L, Trujillo H, et al. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of *Mycobacterium tuberculosis* infection. *Int J Tuberc Lung Dis* 1999;3:138–42.
26. Bartlett JG, Dowel SF, Mandell LA, et al. Practice guidelines for the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2000;31:347–82.
27. Azzimonti-Renzo JC. The agreement between two diagnostic methods in binary cases: a proposal. *Scand J Clin Lab Invest* 2002;62:391–8.
28. Centers for Diseases Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morb Mortal Wkly Rep* 1992;41(RR-17):1–19.
29. Bartlett JG. Pneumonia in the patient with HIV infection. *Infect Dis Clin North Am* 1998;12:807–20.
30. Velásquez G, Mejía P, Restrepo N, et al. Comportamiento de la tuberculosis en pacientes infectados por el Virus de Inmunodeficiencia Humana. *Infectio* 2000;4:32.
31. Programa SIVIGILA. Instituto Nacional de Salud, República de Colombia. Boletín Epidemiológico Semanal 2003; semana epidemiológica No. 11 (marzo 9–15):2–5. Available at: <http://www.col.ops-oms.org/sivigila/2003/BOLE11_03.pdf>. Accessed 29 September 2004.
32. Pan American Health Organization. Epidemiological status of TB. Estimated TB incidence rate (Latin America, 2003). Available at: <<http://www.paho.org/english/hcp/hct/tub/tuberculosis.htm>>. Accessed 29 September 2004.
33. Souweine B, Veber B, Bedos JP, et al. Diagnostic accuracy of protected specimen brush and bronchoalveolar lavage in nosocomial pneumonia: impact of previous antimicrobial treatments. *Crit Care Med* 1998;26:236–44.
34. King JC. Community respiratory viruses in individuals with human immunodeficiency virus infection. *Am J Med* 1997; 102:19–24.
35. Bantar C, Bavestrello L, Curcio D, the ConsenSur Group. Acute community-acquired pneumonia in adults: guidelines for initial antimicrobial therapy based on local evidence from a South American working group (ConsenSur). *J Chemother* 2002; 14(Suppl. 4):1–24.
36. Programa SIVIGILA. Instituto Nacional de Salud, República de Colombia. Boletín Epidemiológico Semanal 2001; semana epidemiológica No. 30, julio 22–8, 2001. p. 1–9. Available at: <http://www.col.ops-oms.org/sivigila/2001/BOLE30_2001.htm>. Accessed 2 August 2005.
37. Boeckh M, Boivin G. Quantitation of Cytomegalovirus: methodologic aspects and clinical applications. *Clin Microbiol Rev* 1998;11:533–54.
38. Sarmiento M, Castaño ME, Bedoya VI, et al. Utility of antigenemia test for cytomegalovirus detection in hematopoietic transplant recipients: report of experiences from Medellín, Colombia. *Bone Marrow Transplant* 2003;32:119–20.
39. Chevret S, Scieux C, Garrait V, et al. Usefulness of the cytomegalovirus antigenemia assay for predicting the occurrence of CMV disease and death in patients with AIDS. *Clin Infect Dis* 1999;28:758–63.